Peripheral Glial Cell Line–Derived Neurotrophic Factor Facilitates the Functional Recovery of Mechanical Nociception Following Inferior Alveolar Nerve Transection in Rats

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Aims: To identify endogenous sources of glial cell line–derived neurotrophic factor (GDNF) at the injury site following inferior alveolar nerve transection (IANX) and to determine whether GDNF signaling promotes the recovery of orofacial pain sensation. **Methods:** Nociceptive mechanical sensitivity of the facial skin was assessed following IANX (n = 10) or sham operation (n = 7). GDNF-positive cells were identified and the amount of GDNF measured in the injured region of IANX rats (n = 10) and in sham rats (n = 10). The number of trigeminal ganglion neurons with regenerated axons and the nociceptive mechanical sensitivity after continuous GDNF administration at the injury site were also assessed in IANX (n = 28) and sham (n = 12) rats. The effect of GDNF neutralization on nociceptive mechanical sensitivity at the injury site was evaluated using a neutralizing antibody (GFRα1 Nab) in four groups: IANX + phosphate-buffered saline (PBS) (n = 6); sham (n = 12); IANX + GDNF (n = 12); and IANX + GDNF + GFRα1 Nab (n = 12). Statistical analyses included one-way and two-way repeated measures analysis of variance followed by post hoc tests or unpaired *t* tests. The threshold for statistical significance was set at $P < .05$. **Results:** Nociceptive mechanical sensitivity was lost over the 5 days following IANX and was recovered by day 13. GDNF was expressed in infiltrating inflammatory cells and had enhanced expression. GDNF administration enhanced axonal regeneration and recovery of nociceptive mechanical sensitivity. GDNF neutralization inhibited the recovery of nociceptive mechanical sensitivity after IANX. **Conclusion:** GDNF signaling at the injury site facilitates the functional recovery of mechanical nociception following IANX and is an attractive therapeutic target for the functional disturbance of pain sensation. *J Oral Facial Pain Headache 2018;32:229–237. doi: 10.11607/ofph.2052*

Keywords: *GDNF family receptor alpha, glial cell line–derived neurotrophic factor, inferior alveolar nerve injury, mechanical nociception, nerve regeneration*

Injury of the inferior alveolar nerve (IAN), a branch of the mandibular nerve, occasionally occurs as a complication of orofacial surgery, such as impacted tooth extraction, dental implant placement, mandibular fracture, or endodontic treatment.[1–3] IAN injury can induce various sensory disturbances in orofacial sites innervated by the IAN that necessitate therapeutic intervention, including vitamin preparations and photobiomodulation.[4,5] Nonetheless, the available individual treatments are not very effective, and so there is a clinical need for the development of novel therapeutic interventions for sensory disturbances following IAN injury.

The glial cell line–derived neurotrophic factor (GDNF) family consists of GDNF, neurturin, artemin, and persephin.[6] The receptor tyrosine kinase RET is a low-affinity receptor for GDNF family ligands, and there are four GDNF family receptor alpha (GFRα) receptors (GFRα1 to GFRα4) that act as high-affinity receptors for each GDNF family ligand, respectively.[7,8] GDNF is upregulated in several cell types, including macrophages, microglia, and Schwann cells, proximal to nerve injury.[9–11] Facial nerve injury causes the activation of purinergic signaling in Schwann cells, which induces GDNF gene transcription via c-Jun,

protein kinase C, and protein kinase D.[12] Moreover, GDNF delivery to the injury site enhances morphologic axonal regeneration and myelination following peripheral nerve injury.[13–16] However, the effect of GDNF signaling in the region of peripheral nerve injury on functional axonal regeneration (ie, the recovery of sensory function) is not well understood.

The aim of the present study was to identify endogenous sources of GDNF at the injury site following IAN transection (IANX) and to determine whether GDNF signaling promotes the recovery of orofacial pain sensation.

Materials and Methods

Animals
Male Sprague-Dawley rats (N = 119; 200 to 250 g; Japan SLC) were housed in a temperature-controlled room (23°C) on a 12-hour light-dark cycle (lights on at 07:00) with ad libitum access to food and water. This study was conducted in accordance with the guidelines of the International Association for the Study of Pain[17] and approved by the Animal Experimentation Committee at Nihon University (AP15D012). All efforts were made to minimize animal suffering and reduce the number of animals used.

Inferior Alveolar Nerve Transection
All surgical procedures were performed under deep anesthesia induced with a combination of butorphanol tartrate (2.5 mg/kg; Meiji Seika Pharma), midazolam (2.0 mg/kg; Sandoz), and medetomidine (0.375 mg/kg; Zenoaq). In the rats that underwent IANX (n = 78), an incision was made on the skin of the left cheek and in the left masseter muscle, and the mandibular bone surface was superficially drilled using a low-speed round bur to expose the IAN. The left IAN was completely transected with microscissors at 5 mm distal to the mental foramen, and the transected IAN was re-placed into the mandibular canal. Skin and muscle incisions were sutured with 5-0 silk. As a control, skin incision, muscle dissection, and bone drilling were performed without IANX in 41 rats (sham). Body temperature was maintained during all surgical procedures, and animals were isolated until they recovered from anesthesia.

Nociceptive Mechanical Sensitivity
IANX (n = 10) and sham (n = 7) rats were anesthetized using 2% isoflurane (Mylan) until weakening of the hind limb flexion reflex was observed to ensure an adequate level of anesthesia maintenance. The flexion reflex was induced by applying noxious pressure to the hind paw following cessation of the isoflurane supply, and the threshold intensities for evoking the

head withdrawal reflex elicited by mechanical stimulation of the left facial skin above the mental foramen were measured using an electronic von Frey anesthesiometer (0 to 200 g [cutoff 200 g], 10 g/s; Bioseb), as previously described.[18–20] Graded mechanical stimuli were applied at 1-minute intervals. Rats were free to escape the mechanical stimulation. The lowest mechanical intensity that evoked a nocifensive reflex (head withdrawal) was noted as the mechanical head withdrawal threshold (MHWT). The average MHWT was determined based on responses to three stimulus applications of the same mechanical intensity. This procedure was conducted every other day until day 13 post-IANX (Fig 1a). All measurements of facial mechanical sensitivity were conducted under blinded conditions.

Drug Delivery to the Transected IAN
Before surgery, fibrin gels were prepared by mixing fibrinogen (40 μL, 75 to 115 mg/mL) and thrombin (40 μL, 500 IU/mL), which were obtained from BeriplastP (CSL Behring) and resuspended in accordance with manufacturer specifications. The fibrin gels were loaded with MedGel (MedGEL), a gelatin-based hydrogel used for the sustained release of bioactive substances. A volume of 20 μL of GDNF (1.25 μg; Sigma-Aldrich) diluted in 0.01 M phosphate-buffered saline (PBS) or 20 μL of vehicle was mixed into the fibrin gels loaded with MedGel. GDNF- or vehicle-containing MedGel were stored overnight at 4°C. The volumes and concentrations of fibrinogen, thrombin, and GDNF were determined based on a previous report.[21] Then, under deep anesthesia, the fibrin gels loaded with MedGel were placed at the cut ends of the transected IAN immediately after IANX prior to skin and muscle closure. In 12 rats from the GDNF-treated IANX group (n = 24), a GFRα-1/GDNF Rα-1 neutralizing antibody (GFRα1 Nab) (10 μL; 0.1 mg/ml; R&D Systems) dissolved in 0.01 M PBS was transcutaneously injected at the cut ends of the transected IAN every day until day 13 post-IANX under light anesthesia with 2% isoflurane. Measurements of MHWT were conducted every other day until day 13 post-IANX in the IANX + PBS (n = 6), IANX + GDNF (n = 12), IANX + GDNF + GFRα1 Nab (n = 12), and sham (n = 12) groups (Fig 1b).

Immunohistochemistry for the Transected IAN
On day 5 post-IANX or sham procedure, rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal [ip]) and then transcardially perfused with cold saline followed by 4% paraformaldehyde fixative in 0.1 M phosphate buffer (4°C, pH 7.4). The mandibular bone containing the transected IAN was dissected and immersed in the
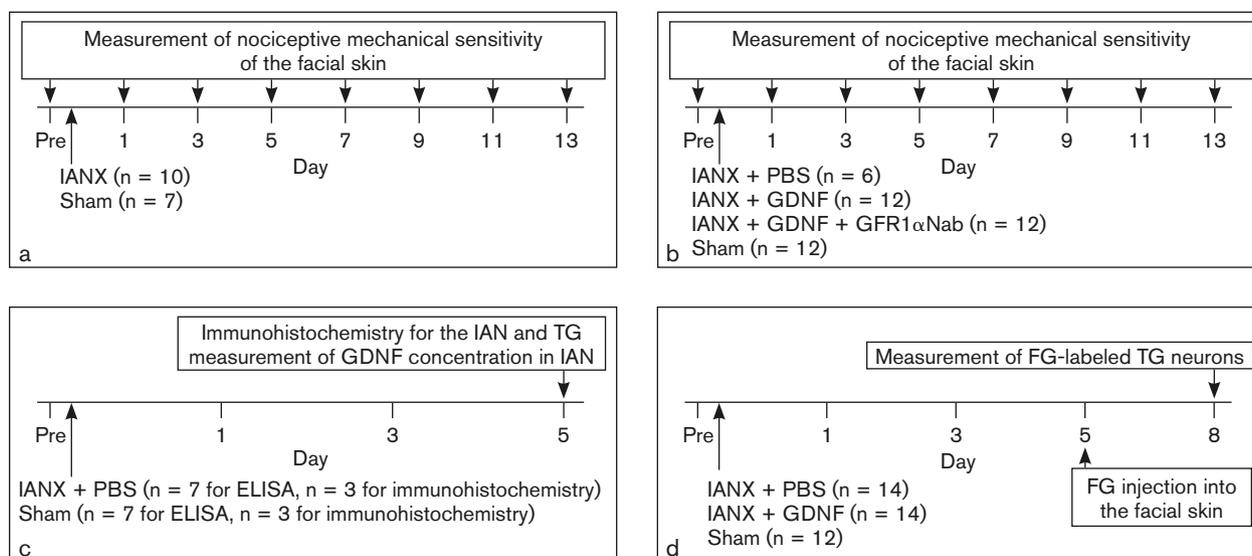
Fig 1 Schematic diagram describing the different experimental groups. **(a)** Measurement of the nociceptive mechanical sensitivity of the facial skin in the IANX and sham groups. **(b)** Measurement of the nociceptive mechanical sensitivity of the facial skin in the sham, IANX + PBS, IANX + GDNF, and IANX + GDNF- + GFRα1 Nab groups. **(c)** Measurement of GDNF concentration in the IAN and immunohistochemistry of the IAN and TG in the sham and IANX + PBS groups. **(d)** Measurement of FG-labeled TG neurons in the sham, IANX + PBS, and IANX + GDNF groups.

same fixative for 4 hours at 4°C. The mandibular bone was decalcified with K-CX (Falma) for 72 hours and neutralized under running water overnight. Then, samples were placed in a 0.01-M PBS solution containing 20% sucrose for 12 hours for cryoprotection and subsequently embedded in TissueTek (Sakura Finetek) at −20°C. The next day, the mandibular bone was cut in the mesiodistal plane on a cryostat into 10-μm sections. The sections were thaw mounted onto Matsunami adhesive saline (MAS)–coated Superfrost Plus microscope slides (Matsunami) and dried at 23°C. After rinsing with PBS, the sections were incubated with anti-GDNF goat polyclonal antibody (1:100, cat.no. AF-212-NA; R&D Systems) and anti-F4/80 rabbit monoclonal antibody (1:200, cat.no. ab111101; Abcam), anti-CD44 rabbit polyclonal antibody (1:100, cat.no. ab24504; Abcam), or anti-Iba1 rabbit polyclonal antibody (1:100, cat.no. 019-19741; Wako) diluted in 0.01 M PBS containing 4% normal goat serum and 0.3% Triton X-100 (Sigma-Aldrich) overnight at 4°C. After rinsing with 0.01 M PBS, the sections were incubated with Alexa Fluor 488-conjugated donkey anti-rabbit Immunoglobulin G (IgG) (1:200 in 0.01 M PBS; Thermo Fisher Scientific) and Alexa Fluor 568-conjugated donkey anti-goat IgG (1:200 in 0.01 M PBS, Thermo Fisher Scientific) or with Alexa Fluor 568-conjugated donkey anti-mouse IgG (1:200 in 0.01 M PBS, Thermo Fisher Scientific) for 2 hours at 23°C. Finally, sections were rinsed in 0.01 M PBS and coverslipped in mounting medium (Thermo Fisher Scientific). No specific labeling was observed in the absence of primary antibody. GDNF- and CD44-positive cells and GDNF- and Iba1-positive cells at 1 mm proximal to the site of IANX were examined under a fluorescence microscope in IANX + PBS (n = 3) and sham (n = 3) rats (Fig 1c).

Immunohistochemistry for the Trigeminal Ganglion

In the same rats, the trigeminal ganglion (TG) was removed and postfixed in the same fixative for 2 days, then transferred into a 0.01-M PBS solution containing 20% sucrose for 12 hours for cryoprotection. Sections 30 μm thick were cut with a cryostat and thaw mounted onto MAS-coated Superfrost Plus microscope slides (Matsunami) and dried at 23°C. Samples were then incubated with GFRα-1/GDNF Rα-1 polyclonal mouse antibody (1:400 in 0.01 M PBS, cat.no. af560; R&D Systems) overnight at 4°C. After rinsing with 0.01 M PBS, sections were incubated with Alexa Fluor 568-conjugated goat anti-mouse IgG (1:200 in 0.01 M PBS, Thermo Fisher Scientific) for 2 hours at 23°C. Cells showing intensities greater than 2-fold the average background were considered to be immunopositive (Fig 1c). No specific labeling was observed in the absence of primary antibody.

Alternatively, on day 5 post-IANX or sham procedure, a 3% solution of FluoroGold (FG; 4% hydroxystilbamidine) retrograde labeling tracer (Fluorochrome) dissolved in saline (2.5 μL) was injected using a 30-gauge needle into the facial skin under light anesthesia with 2% isoflurane. Three days after the FG injection (day 8 post-IANX), the TG was dissected, cut
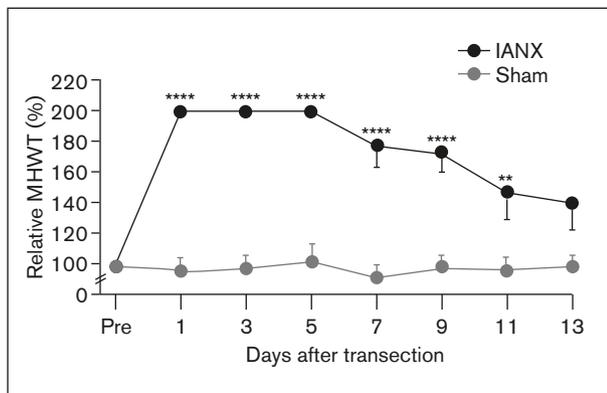
Fig 2 Changes in the MHWT of the facial skin above the mental foramen over 13 days after IANX or sham surgery. Error bars indicate the standard error of the mean. **$P < .01$. ****$P < .0001$. Two-way repeated measures ANOVA followed by Sidak post hoc tests were used (IANX = 10; sham = 7).

into 30-μm sections, and mounted on MAS-coated Superfrost Plus microscope slides as described above. Six sections per TG (every 300 μm) were used for the analysis. A fluorescence microscope (BZ-9000 system; Keyence) was used for the identification and analysis of FG-labeled neurons. Neurons showing intensities greater than 2-fold the average background were considered to be labeled with FG. The mean number of total FG-labeled neurons in six sections was calculated for each group (IANX + PBS [n = 14]; IANX + GDNF [n = 14]; sham [n = 14]). The mean number of FG-labeled neurons classified in accordance with cell area (every 200 μm^2) was calculated and plotted on a size-frequency histogram (Fig 1d).

GDNF Concentration in the Transected IAN
On day 5 post-IANX or sham procedure, rats were deeply anesthetized (5 mg/kg butorphanol tartrate, 4 mg/kg midazolam, and 0.75 mg/kg medetomidine) and transcardially perfused with cold saline. Both the proximal and distal sites of the transected IAN were quickly dissected on ice. Then, tissues were homogenized in 200 μl of lysis buffer (137 mM NaCl, 20 mM Tris-HCl [pH 8.0], 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 1 g/mL leupeptin, and 0.5 mM sodium vanadate) using a tube pestle (Thermo Fisher Scientific) and centrifuged at 15,000 rpm for 10 minutes at 4°C. The supernatants were collected into new tubes, and protein concentrations were determined with a protein assay kit (Bio-Rad). After adjusting the protein concentrations of all samples (IANX + PBS [n = 7]; sham [n = 7]), GDNF concentrations were measured with a GDNF enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech) in accordance with the manufacturer's specifications (Fig 1c).

Statistical Analyses
Data are expressed as the mean ± standard error of the mean (SEM). The estimation of sample sizes was based on previous experience.[22] Two-way repeated-measures analysis of variance (ANOVA) with post hoc Bonferroni tests were used for the behavioral and pharmacologic analyses. Unpaired Student t tests were used to analyze the ELISA. One-way ANOVA with post hoc Holm-Sidak multiple comparisons tests were used for the analysis of FG-labeled neurons in each size-frequency histogram. P values < .05 were considered statistically significant.

Results

**Orofacial Mechanical Sensitivity Following IANX**
The MHWT of the facial skin above the mental foramen following IANX was significantly higher than that following sham surgery from days 1 to 11 (Fig 2); however, this difference disappeared by day 13. The MHWT in the sham group did not change significantly during the experimental period. Sedation and motor deficits were not observed during the experimental period, and there were no pathologic changes in the facial skin (data not shown).

GDNF Expression in the Transected IAN
Many CD44-immunoreactive (IR), F4/80-IR, and Iba1-IR cells expressed GDNF in the transected IAN at day 5 post-IANX (Figs 3a to 3c). Moreover, the amount of GDNF protein in the transected IAN (2.0 ± 0.1 pg/mL) was significantly higher than that in IAN tissues of the sham group (1.6 ± 0.1 pg/mL) (Fig 3d).

Effect of GDNF Administration on IAN Regeneration Following IANX
The presence of FG-labeled neurons in the TG indicated re-innervation of the facial skin above the mental foramen following IANX. Most FG-labeled neurons were localized to the mandibular division of the TG on day 8 after sham surgery or IANX in animals that were treated with PBS or GDNF (Fig 4a). The analysis of cell area revealed that the number of FG-labeled, small-sized (≤ 199 μm^2 and 200 to 399 μm^2 in area) TG neurons was increased in the GDNF-treated IANX group compared to the PBS-treated IANX group (≤ 199 μm^2, PBS: 8.8 ± 2.4, GDNF: 19.9 ± 3.5, $P = .08$; 200 to 399 μm^2, PBS: 16.0 ± 2.6, GDNF: 32.9 ± 3.3, $P = .06$) (Fig 4b). The number of FG-labeled TG neurons (200 to 399 μm^2, 400 to 599 μm^2, 600 to 799 μm^2, and 800 to 999 μm^2 in area) in the sham group was significantly larger than that of the GDNF-treated IANX group.
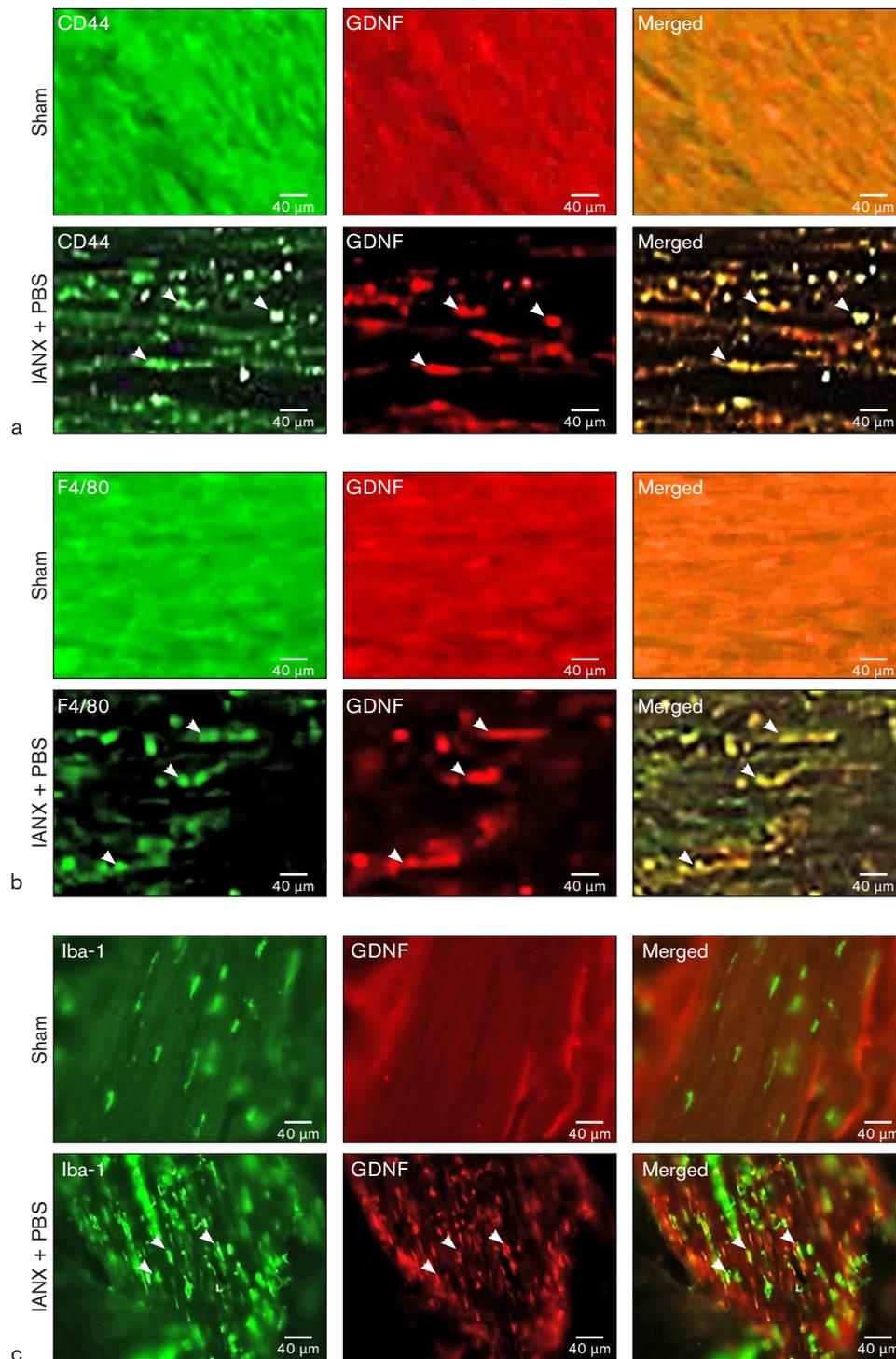
Fig 3 GDNF expression at the site of injury on day 5 after IANX or sham procedure. Photomicrographs of **(a)** CD44-positive and GDNF-positive cells, **(b)** F4/80-positive and GDNF-positive cells, and **(c)** Iba1-positive and GDNF-positive cells at the injury site in the sham and IANX + PBS group. Arrows indicate double-positive cells at the injury site. **(d)** Relative GDNF expression at the injury site on day 5 post-IANX. Error bars indicate the standard error of the mean. **P < .01. Unpaired *t* test was used (n = 7 per group).

GFRα1 Expression in the TG and the Effect of GDNF Treatment on Nociceptive Mechanical Sensitivity Following IANX

GFRα1 was expressed in small- and medium-sized TG neurons on day 5 following IANX or sham surgery with PBS or GDNF treatment (Fig 5a). GDNF treatment significantly enhanced the recovery of MHWT compared to vehicle treatment at day 5 post-IANX (IANX + PBS, 200.0% ± 0.0%; IANX + GDNF, 151.4% ± 13.1%, P < .05) (Fig 5b). Moreover, GDNF-enhanced MHWT recovery was significantly attenuated by continuous GFRα1 Nab treatment at the cut ends of the transected IAN on day 11 post-IANX (IANX + GDNF + GFRα1 Nab, 174.5% ± 11.1%;
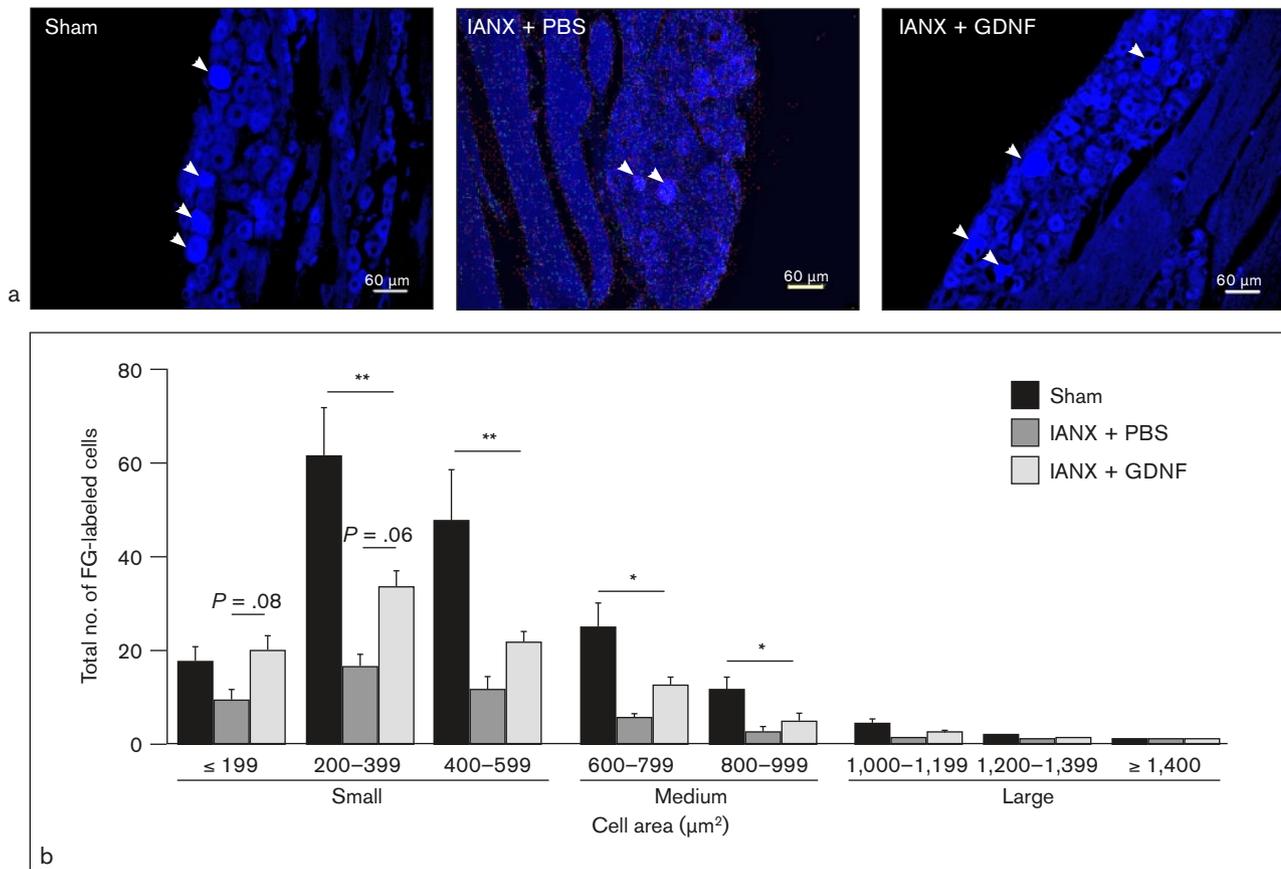
Fig 4 The regenerated IAN innervating the facial skin above the mental foramen on day 8 post-IANX or sham procedure. **(a)** FG-labeled TG neurons on day 8 in the sham, IANX + PBS, and IANX + GDNF groups. Arrows indicate FG-labeled TG neurons. **(b)** Size-frequency distribution of FG-labeled TG neurons on day 8 in the sham, IANX + PBS, and IANX + GDNF groups. Error bars indicate the standard error of the mean. *$P < .05$. **$P < .01$. One-way repeated measures ANOVA followed by Holm-Sidak's multiple comparison post hoc tests were used (sham = 12; IANX + PBS = 14; IANX + GDNF = 14).

IANX + GDNF, 132.4% ± 11.1%, $P < .05$) (Fig 5c). The MHWT in the sham group did not change significantly during the experimental period. Sedation and motor deficits were not detected during the experimental period, and there were no pathologic changes in the facial skin (data not shown).

Discussion

Sensory disturbances such as hypoesthesia and anesthesia occur in the ipsilateral facial skin and teeth following IAN injury.[23,24] In most cases, these sensory disturbances are transient and subside spontaneously in the weeks to months after the injury, although abnormalities can persist in some cases.[25,26] In this study, sensory changes in the facial skin above the mental foramen induced by IANX lasted for the 13 days monitored postinjury. Notably, there was complete anesthesia in the facial skin in the postinjury period, but there were no pathologic changes in the facial skin or motor deficits in the orofacial region.

Generally, tissue injury from a sham procedure, such as the skin incision or muscle dissection, induces mechanical hypersensitivity at the injured site.[19] However, the site at which MHWT was measured was far from the region with tissue injury due to the sham procedure in this study. This indicates that the sham procedure did not influence the mechanical sensitivity of the left ipsilateral facial skin. Therefore, IANX provides a useful model of sensory disturbance in patients with a history of traumatic injury to the IAN.

Peripheral nerve injury produces the rapid upregulation of calprotectin, an S100A8/A9 heterodimer, which in turn stimulates the release of several cytokines and chemokines.[27] Signaling molecules like tumor necrosis factor alpha, vascular cell adhesion protein 1, and interleukin-15 promote the infiltration of immune cells.[27–29] Indeed, preclinical and clinical studies have described the accumulation of inflammatory cells such as macrophages, neutrophils, and T lymphocytes in regions of peripheral nerve injury.[30,31] It has previously been reported that activated macrophages at the injury site are the predominant
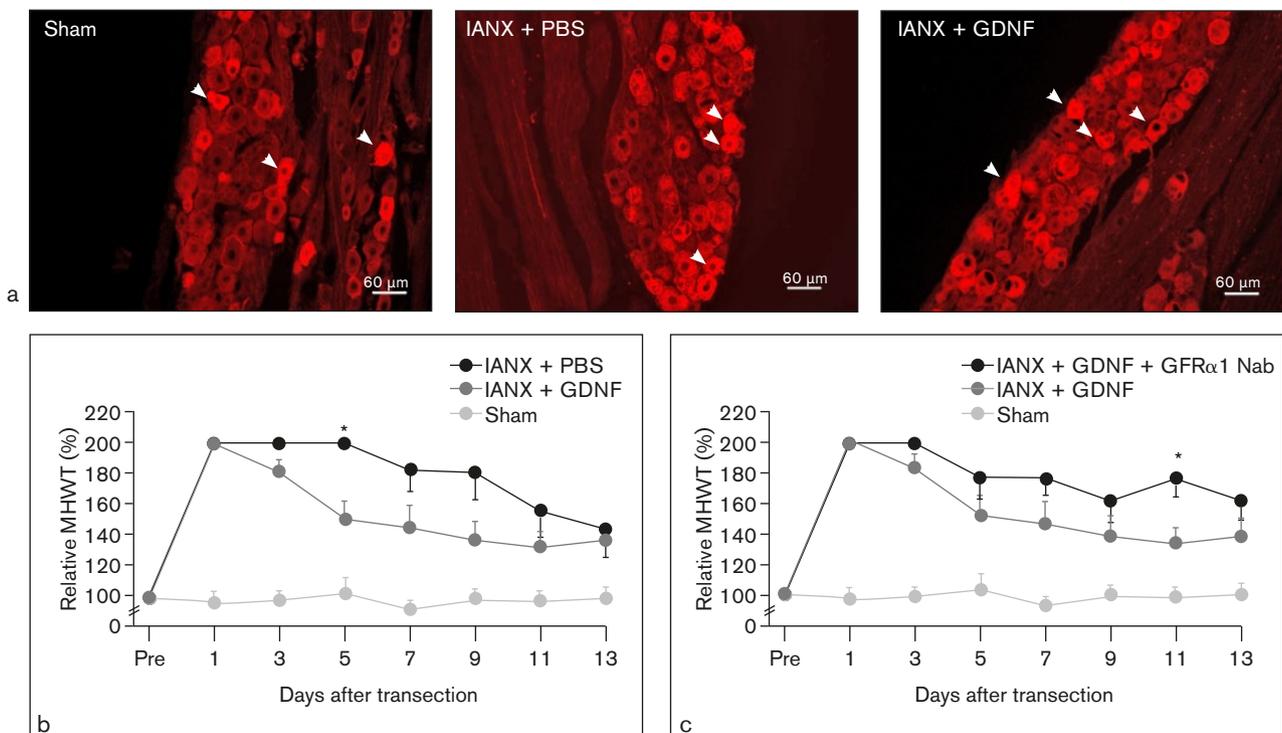
Fig 5 Effect of local GDNF treatment on changes in the MHWT of the facial skin after IANX or sham procedure. **(a)** GFRα1 expression in the TG on day 8 in the sham, IANX + PBS, and IANX + GDNF groups. Arrows indicate GFRα1-positive TG neurons. **(b)** Changes in the MHWT of the facial skin over 13 days in the sham, IANX + PBS, and IANX + GDNF groups. **(c)** Changes in the MHWT of the facial skin over 13 days in the sham, IANX + GDNF, and IANX + GDNF + GFRα1 Nab groups. Error bars indicate the standard error of the mean. *$P < .05$ vs IANX + GDNF group. Two-way repeated measures ANOVA followed by Sidak post hoc tests were used (IANX + GDNF = 12; IANX + PBS = 6; IANX + GDNF + GFRα1 Nab = 12; sham = 12).

sources of GDNF after nerve injury.[32,33] In the present study, numerous macrophages and neutrophils expressing GDNF had infiltrated the injury site by day 5 post-IANX, whereas these cells were virtually nonexistent at the site of sham injury. GDNF was more abundantly expressed at the injury site in IANX rats than in sham-operated rats. These results, as well as those from previous reports, suggest that GDNF concentration at the injury site was increased following release from infiltrated macrophages as a consequence of IANX. It is possible that infiltrated neutrophils are also a source of GDNF, which increases at the injury site in IANX.

In previous studies, the downstream effects of GDNF were attributed to GFRα1 binding coupled with a signal-transducing domain of RET, which stimulates intracellular cascades such as c-Jun N-terminal kinase (JNK),[34] mitogen-activated protein kinase (MAPK),[35] and extracellular signal-regulated kinase (ERK).[36] For example, the Na-K-2 Cl type 1 co-transporter is induced by JNK signaling after sensory neuron injury and promotes the regeneration of myelinated sensory axons after sciatic nerve transection.[37] p38 MAPK signaling enhances neurite outgrowth in PC12 cells in vitro, suggesting that p38

MAPK signaling plays a role in axonal regeneration.[38] The ERK pathway also mediates axonal elongation via several neurotrophins, including GDNF.[39,40] In particular, substantial ERK activation is required for the fast arrangement of growth cones in response to local extracellular signaling and is also fundamental for growth cone turning governed by netrin signaling.[41] It is well known that Schwann cells, in addition to regenerating nerve endings, express GFRα1 after peripheral nerve injury.[42,43] GDNF promotes Schwann cell differentiation through the phosphorylation of Fyn, a Src family tyrosine kinase, and leads to the acceleration of peripheral nerve regeneration by reinforcing myelination.[42] Small- and medium-diameter sensory neurons correspond to unmyelinated C fibers and myelinated Aδ fibers, respectively, most of which play critical roles in nociception. Alternatively, large-diameter sensory neurons correspond to myelinated Aβ-fibers, which are responsible for transmitting innocuous mechanical input.[44,45] Small- and medium-diameter sensory neurons (in particular small-diameter sensory neurons) predominantly express ion channels that include transient receptor potential (TRP) vanilloid 1 (TRPV1), TRPV4, and TRP ankyrin 1 (TRPA1), which play important roles

in mechanical nociception.[46,47] Many small- and medium-sized TG neurons expressing GFRα1 after IANX that were likely to be regenerating nerve endings were observed in the present study. The cell-area analysis revealed that the GDNF delivery to the injury site markedly expedited the regeneration of these small-sized TG neurons, suggesting that GDNF-GFRα1 interaction was responsible for the beneficial effects of GDNF treatment. This hypothesis is supported by the observation that GFRα1 antibody attenuated the beneficial effects of GDNF treatment on sensory recovery. Together with previous studies, these results indicate that GDNF released from infiltrating macrophages and neutrophils may promote the regeneration of nociceptive unmyelinated and thinly myelinated axons through GFRα1 binding on injured sensory nerve endings. Further, it is also possible that GDNF signaling in Schwann cells accelerates myelination to promote nerve regeneration, leading to the functional recovery of mechanical nociception after IAN injury.

Only male rats were used in this study. Earlier studies have indicated that estradiol stimulates GDNF expression in the central nervous system.[48] This may indicate that peripheral expression of GDNF, which accelerates myelination to promote nerve regeneration, is also influenced by female hormones. In addition, it is well known that some small afferent nerves are thermosensitive and nonnociceptive.[49,50] GDNF delivery to the injury site accelerates the regeneration of small TG neurons following IANX. This suggests that local GDNF delivery may not accelerate the functional recovery of mechanical nociception, but does so for thermal sensation or nonnociceptive information following peripheral nerve injury. Further studies are thus required to investigate this phenomenon.

Conclusions

Sustained GDNF administration achieved by implantation of loaded MedGel at the injury site significantly accelerated the functional recovery of mechanical nociception in the facial skin following IAN injury. From a clinical perspective, MedGel, a gelatin-based hydrogel utilized for the sustained release of bioactive substances, may represent an attractive therapeutic option for accelerating the functional recovery of normal pain sensation in the facial skin following IAN injury.

Acknowledgments

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